Identification of AMPK Phosphorylation Sites Reveals a Network of Proteins Involved in Cell Invasion and Facilitates Large-Scale Substrate Prediction.

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Public Summary:
Within a cell, a protein called AMP-activated protein kinase (AMPK) senses when the cell is low on energy and then helps modify the cell's energy use by inhibiting processes that consume energy and activating processes that provide energy. AMPK regulates these processes by placing a chemical tag, called phosphorylation, on the responsible proteins. This phosphorylation then changes the substrate protein's function. Historically, studies focused on the role of AMPK in energy conservation, but recent studies have revealed that AMPK is involved in many different cellular processes, such as cell division. In addition, AMPK is involved in development as well as many diseases like cancer and aging, but when compared to energy conservation, it is less well understood how AMPK regulates these processes. In this study, we wanted to identify new protein substrates of AMPK as well as the exact location of the phosphorylation site, as this will help determine how AMPK is changing the activity of the protein substrate. Since many proteins use phosphorylation tags to regulate their own substrates, we modified AMPK to use a slightly different form of this tag that could be distinguished from normal forms of the tag. Using this approach, we found many novel AMPK substrates, and some of the new substrates further connect AMPK to energy-related processes. However, a large number of the new substrates were involved in different ways that a cell moves through its environment- by "crawling," "sticking," or "digging" to or through extracellular scaffolds or other cells. When we studied the function of AMPK's regulation of one such protein, called NET1A, we found that AMPK phosphorylation of NET1A prevented the cell from digging into and degrading the extracellular scaffold it rested on. This process, called extracellular matrix degradation, is involved both in development and in the early stages of some cancer metastases. These findings indicate that AMPK may help prevent cancer cells from metastasizing, in part through phosphorylation of the novel substrate NET1A. The identification of many direct AMPK phosphorylation sites facilitated the development of an algorithm to predict additional AMPK substrates in pre-existing datasets, as AMPK places the phosphorylation tag at sites surrounded by similar sequences. As AMPK is important to both normal developmental as well as many pathological processes like aging and cancer, the novel AMPK substrates identified in this study may provide potential targets for therapeutic strategies.

Scientific Abstract:
AMP-activated protein kinase (AMPK) is a central energy gauge that regulates metabolism and has been increasingly involved in non-metabolic processes and diseases. However, AMPK's direct substrates in non-metabolic contexts are largely unknown. To better understand the AMPK network, we use a chemical genetics screen coupled to a peptide capture approach in whole cells, resulting in identification of direct AMPK phosphorylation sites. Interestingly, the high-confidence AMPK substrates contain many proteins involved in cell motility, adhesion, and invasion. AMPK phosphorylation of the RHOA guanine nucleotide exchange factor NET1A inhibits extracellular matrix degradation, an early step in cell invasion. The identification of direct AMPK phosphorylation sites also facilitates large-scale prediction of AMPK substrates. We provide an AMPK motif matrix and a pipeline to predict additional AMPK substrates from quantitative phosphoproteomics datasets. As AMPK is emerging as a critical node in aging and pathological processes, our study identifies potential targets for therapeutic strategies.

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